Journal of Forestry Research (2011) 22(4): 611–614
DOI 10.1007/s11676-011-0204-6

ORIGINAL PAPER

Seed storage behavior of *Knema attenuata*, an endemic species of Western Ghats, India

Vinayachandra • K. R.Chandrashekar

Received: 2010-11-18; Accepted: 2011-05-02
© Northeast Forestry University and Springer-Verlag Berlin Heidelberg 2011

Abstract: We performed desiccation and storage trials to better understand storage behavior of *Knema attenuata* seeds. Mature seeds with moisture content (MC) of 31% exhibited 73% germination. During the period of desiccation (open lab condition) seeds with MC 23% showed 40% germination. After further drying to MC 21% germination was reduced to 16%. Complete loss in viability resulted when seed moisture was reduced to 18%. The seeds stored at -10°C, 0°C, 10°C and 28±2°C (open lab condition) lost their viability within 10 days. Seeds stored in sealed polythene bags and moist sand retained viability for more days than did seeds stored under all other storage conditions. Sensitivity of seeds to lower temperature and desiccation suggest that the storage behavior of *K. attenuata* seeds is recalcitrant. Seeds stored in moist conditions can, at best, be stored for a period of two months.

Keywords: desiccation; germination; *Knema attenuata;* recalcitrant; storage; viability

Introduction

Information on seed longevity, desiccation, and freezing sensitivity is a prerequisite for conserving plant species that generally produce non-orthodox seeds. Seed longevity and storage behavior of many wild and semi-domesticated species from tropical regions has been documented (Hong and Ellis 1996). Based on seed storage behavior, three categories of seeds viz., orthodox, recalcitrant, and intermediate or orthodox with limited desiccation ability are recognized and widely used (Hong and Ellis 1996; Schmidt 2000).

Knema attenuata (Hook. f. & Thomson) Warb. (Myristicaceae) is a medium sized tree endemic to southern India and frequent in Western Ghats, extending from Konkan southwards to Maharashtra, Goa, Karnataka, Tamil Nadu, and Kerala. It provides one of the ingredients of 'Ashwagandadhi nei' (medicated ghee) that is used for treatment of spleen disorders, breathing disorders and tastelessness (Ravikumar and Ved 2000). Conventionally, the trees are propagated by seeds. *Knema* seeds remain viable only for a week at normal dry conditions (unpublished data). Little information is available on the storage and germination of these seeds. Thus we undertook investigation of the germination, desiccation, and viability of Knema seeds in storage to strengthen the strategies for the conservation of this species.

The online version is available at http:// www.springerlink.com

Vinayachandra • K. R. Chandrashekar (✉)
Department Applied Botany, Mangalore University, Mangalagangothri – 574199, Mangalore, Karnataka, India. Email: konambi@yahoo.com

Responsible editor: Hu Yanbo

Materials and methods

Mature, split, and aril-exposed fruits of *K. attenuata* were manually harvested during June 2009 from trees selected on the basis of their fruit production and numbers of emerged seedlings beneath them. Our study site was the Charmady forest of Dakshina Kannada district, Karnataka, India. Seeds were transported to the laboratory in polythene bags. Fruit rinds and arils were removed and cleaned. Seed samples without any apparent physical damage or insect infestation were selected for the experiment.

Germination test

To learn the role of testa in seed germination we germinated seeds with and without testa in sand beds in accordance with the International Seed Testing Association procedures (ISTA 1991). We scored germination according to the emergence of radicles (2 cm in length) and expressed this as the percentage of seeds germinated.

Desiccation study

We spread about 400 seeds evenly on plastic trays and allowed them to dry at open room conditions. Samples of 75 (25 × 3) seeds were removed at intervals of two days and set to germinate following ISTA (1991) procedures. Moisture content (MC) of whole seeds was determined following the low constant oven drying method (ISTA 1991). We randomly selected five seeds

and cut them into quarters that we dried at 103ºC for 17 h in a hot air oven. Seed MC was calculated on a fresh mass basis.

Storage trials

Storage trials were conducted with de-arillated seeds in sealed polythene bags (25 μ, 25 × 20 cm) kept at -10ºC, 0ºC, and 10ºC laboratory conditions (28±2ºC) and in polythene jars containing moist sand (26±2ºC). Viability of the stored seeds was monitored by germination tests following the method described earlier at the intervals of 10 days.

Tetrazolium chloride test

Seed viability was also evaluated by treating dissected embryos (five embryos in each case) with 1% 2,3,5 –triphenyl tetrazolium chloride solution (TZ) for 14 h at 32ºC in darkness. Staining patterns were observed under a dissection microscope (ISTA 1991).

Statistical analysis

The statistical significance of the results on the storage studies were tested using analysis of variance (ANOVA), AGRES version.7.01. Since some of the observations were zero, square root transformations were made prior to applying the ANOVA test. Statistical significance was set at $\alpha = 0.05$.

Results and discussion

Seeds were solitary, egg-shaped and enclosed within a crimson coloured fleshy aril. A brown papery envelope was present inside the aril followed by a dark testa with a thickness of 0.5±0.04 mm. Embryos of 3.9±0.25 mm length were located at the border end of the ruminate endosperm of 23±1.3 mm in length and 14.6±0.7 mm width towards the peduncle end.

Hypogeal germination was observed in *K. attenuate*, similar to that in other Myristicaceae species. Seeds without testa started germination after 40 days while seeds with testa took 42 days to start germination (Fig.1). Delayed germination was observed in seeds with testa up to 55 days after sowing compared to the seeds without testa. The delay of germination in the seeds with testa indicated the physical dormancy of these seeds. The dormancy of seeds up to 40 days may be morphophysiological dormancy. Morphophysiological dormancy has been observed in other *Knema* species such as *K. furfuracea, K. kurina, K. stenophylla* and *K.* curtisti (Baskin and Baskin 2001). Ng (1978) reported that *Myristica crassa* and *Myristica malaccensis* have underdeveloped embryos that cause morphophysiological dormancy. Kumar et al. (2002), working with seeds of *Myristica malabarica*, observed a significantly faster rate of germination in seeds without testa and inferred that testa physically impedes seed germination. Maximum germination (72%) was observed at the end of day 80 irrespective of presence or absence of testa (Figure 1).

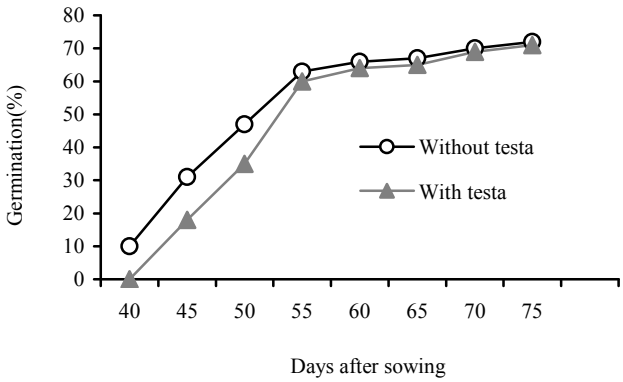

Fig. 1 Germination of *Knema attenuata* seeds with and without testa.

Germination percentage declined from 73% to 16% after six days of desiccation (Table 1). The initial MC was 31% and declined with increasing length of the desiccation period. MC dropped to 18% after 8 days of desiccation and seeds failed to germinate. The time required for maximum germination also increased with the period of desiccation.

Table 1. Effect of desiccation on germination of *Knema attenuata* seeds. (Mean±SD, *n*=3)

Period of desiccation (hour)	Germination (%)	Moisture Content (%)	Time taken for maximum germination (Days)	TZ staining pattern
0	73±2.5	31.1±0.8	72±3.5	Endosperm-red; Embryo-red
48	68±5.3	27.6±0.6	72±2.4	Endosperm-pale red; Embryo-red
96	40±2.5	23.6±0.7	75±2.5	Endosperm-pale red with unstained margins; Embryo-pale red
144	16±3.5	21.2±0.9	86±2.0	Endosperm- pale red withunstained margins; Embryo-pale red patches
168	0	18.2±0.9	N.A	Unstained
CD(0.05)	5.9	2.6	4.38	
P value	0.000	0.000	0.000	

As the MC of *K. attenuata* (31%) gradually declined to the critical level of 21%, the germination percentage decreased to 16% and the germination period lengthened. Seeds of *M. malabarica* at 27% MC lost viability within a week in open dry condition (Kumar et al. 2002). Decocted seeds of *M. fragrans* at 49% MC lost viability within five days (Madhusudanan and Babu 1994). Delayed germination indicated the loss of seed vigour as reported for *Aporusa lindleyana*, which took 14 more days to germinate when MC was reduced from 40-33% (Kumar et al. 1996). The critical moisture content (CMC) of seeds varies be-

tween species, but it is relatively high for recalcitrant seeds, typically ranging from 12%−31% (Roberts 1973). The seeds of two dipterocarps, *Hopea parviflora* and *H. ponga*, are considered to be recalcitrant, having high critical moisture levels of 28% for *H. ponga* and 26% for *H. parviflora* (Rajeshwari Dayal and Kaveriappa 2000). In *Heavea brasiliensis* CMC was around 15%−20% (Chin et al. 1981) and in *Myristica malabarica* CMC values ranged from 14%−27%. In the present study, *K. attenuata* seeds completely lost viability at MC levels below 21%.

Seeds stored at -10°C, 0°C, 10°C, and 28±2°C (open lab condition) did not germinate and embryos appeared to be light brown (Table. 2). The seeds did not take up TZ stain. Seeds stored at 28±2ºC in poly bags registered 40% germination up to one month but lost viability after two months of storage. Seeds started sprouting after 40 days of storage, vigorous growth of mycelia was observed, radical tips became blackened due to necrosis and regeneration was difficult. Seeds stored in plastic jars with moist sand registered 27% germination for up to two months. MC differed only slightly in seeds stored at different temperature conditions, except for seeds stored in open lab condition where MC dropped to 16%. There was a slight reduction in MC during the course of storage. The accumulation of respiratory moisture during storage might be the reason for observed increase in moisture content of stored seeds in sealed polythene bags. Similar observations were reported for seeds of *Aporusa lindleyana* (Kumar et al. 1996) and *Myristica malabarica* (Kumar et al. 2002).

Recalcitrant seed of *Myristica fragrans* at MC above 45% retained viability for a longer period at 5ºC in sealed transparent polythene packs than did seed stored at room temperature (Sangakkara 1993). The low temperature sensitivity of *K. attenuata* seed was evidenced by loss of viability at -10ºC, 0ºC, and 10ºC within 10 days of storage (Table 2). The failure of high moisture content seeds to survive subfreezing temperatures reported here confirms the results obtained for desiccation–sensitive (Jorgensen 1990; Wesley-Smith et al. 1992) and desiccation–tolerant seeds (King and Roberts 1980; Hong and Ellis 1992). Germination percentages of seeds stored in polythene bags at lab conditions (28±2ºC) declined to 40% as MC declined to 31% in one month. This might be due to sensitivity to desiccation as well as an ageing effect. Desiccation–sensitive seeds have been damaged if dried to levels below critical moisture content (Chaitanya and Naithani 1998; Chaitanya et al 2000b). Relatively high critical moisture content (>30%) was reported for seeds of several desiccation–sensitive tree species (Berjak et al. 1989; Tompsett 1992; Chaitanya and Naithani 1994; Chaitanya et al. 2000a; Chaitanya et al. 2000b). Radicle lengths increased gradually over the course of storage among seeds stored in moist sand/PJ at room temperature (28±2ºC). This was accompanied by plumule initiation and root tip necrosis. Pammenter et al (1994) reported that the germinating recalcitrant seeds in storage are exposed to initially mild but increasingly severe water stress that brings about deterioration. Though the temperature-induced seedling death involved necrosis of the root, at 15ºC, *Symphonia globulifera* seedlings could be stored up to seven months (Corbineau and Come 1986).

Table 2. Effect of different storage conditions on longevity of the *Knema attenuata* seeds (mean ± SD, n=3)

Storage condition	Duration (Days)	Moisture content (%)	Germination (%)	Morphological features
-10°C/Pb	10	31.6±0.9	0	Endosperm & Embryo pale cream.
0°C/Pb	10	33.8±2.5	0	Endosperm & Embryo pale cream.
10°C/Pb	10	30.3±3.6	0	Endosperm & Embryo pale cream.
	10	33.5±0.8	68±4.7	Endosperm & Embryo White.
Moist sand/PJ	30	36.4±0.8	50±7.8	Endosperm & Embryo White.
	60	36.61±1.3	27±4.0	Radicle and plumule initiated Radicle length: 5.5cm.
	10	31.8±1.6	70±1.1	Endosperm & Embryo White.
Room 28±2°C/Pb	30	31.4±1.4	40±6.4	Endosperm white & Embryo pale cream, Mycelial growth started.
	60	29.48±0.6	7±2.0	Radicle initiated, tip necrosis.
(Control) Room open 28±2°C	10	16.0±2.0	0	Endosperm & Embryo pale Brown.
CD(0.05)		3.83	6.25	
P value		0.000	0.000	

Pb - Polythene bags; PJ - Polythene Jars

Conclusions

On the basis of results reported here, *K. attenuata* seeds may be classified as true recalcitrants. More work is needed to understand the type of dormancy in *K. attenuata* seeds. This study has shown that *K. attenuata* seeds can, at best, be stored for up to two months in moist sand. Long term storage of *Knema* seeds will only prove possible when methods are devised for blocking germination or growth without leading to excessive dehydration or risks of chilling injury. One approach could involve the use of solutions of suitable osmotic pressure. Another might use cryoprotective agents to enable seeds or seedlings to withstand lower storage temperatures.

Acknowledgements
We gratefully acknowledge Prof. T.P.M. Pakkala for help in statistical analyses. One of the authors (Vinayachandra) is thankful to BRNS for financial assistance in the form of a fellowship.

References

Anil Kumar C, Babu KP, Krishnan PN. 2002. Seed storage and viability of *Myristica malabarica* Lam. an endemic species of Southern Western Ghats (India). *Seed Sci Technol,* **30**: 651−657.

Anil Kumar C, Jacob T, Pushpangadan P. 1996. Storage and germination of seeds of *Aporusa lindleyana* (wight) Baillon an economically important plant of Western Ghats (India). *Seed Sci & Technol,* **25**: 1−6.

Baskin C, Baskin JM. 2001. Seeds: Ecology, Biogeography and Evolution of Dormancy and germination. United States: Academic press, p.247.

Berjak P, Farrant JM, Mycock DJ, Pammenter NW. 1989. Homoiohydrous (recalcitrant) seeds: the enigma of their desiccation sensitivity and state of water in axes of *landolphia kirkii Dyer*. *Planta*, **186**: 249−261.

Chaitanya KSK, Naithani SC. 1994. Role of superoxide, lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability in seeds of *Shorea robusta* Gaertn. *New Phytol,* **126**: 623−627.

Chaitanya KSK, Naithaniand R, Naithani SC. 2000b. Ascorbic acid metabolism in ageing recalcitrant sal (*Shorea robusta* Gaertn.f.) seeds. *Indian Journal of Experimental Biology,* **38**: 1031−1035.

Chaitanya KSK, Keshavakanth S, Naithani SC. 2000a. Changes in total protein and rotease activity in sal (*Shorea robusta*) seeds. *Silva Fennica,* **34**: 71−77.

Chaitanya SK, Naithani SC. 1998. Kinetin mediated prolongation of viability in recalcitrant sal (*Shorea robusta* Gaertn. f.) seeds at low temperature: Role of kinetin in delaying membrane deterioration during desiccation induced injury. *J Plant Growth Regulation*, **17**: 63−69.

Chin HF, Aziz M, Ang BB, Hamsah S. 1981. The effect of moisture content and temperature on the ultra structure and viability of seeds of *Hevea brasiliensis*. *Seed Sci & Technol,* **9**: 411−422.

Corbineau F, Come D. 1986. Experiments on the storage of seeds and seedlings of *Symphonia globulifera* L.f.(Guttiferae). *Seed Sci & Technol,* **14**: 585−591.

Hong TD, Ellis RH. 1996. A protocol to determine seed storage behavior. *IPGRI Technical Bulletin No. 1*. International Plant Genetic Resources Institute, Rome, Italy.

International Seed Testing Association. 1999. *Trees and Shrub seeds handbook*. Zurich: International seed testing association,.

Jorgenson J. 1990. Conservation of valuable gene resources by cryopreservation in some forest tree species. *J Plant Physiol,* **136**: 373−376.

King MW, Roberts EH. 1980. Maintenance of recalcitrant seeds in storage. In: Chin H.F. and E.H. Roberts (Ed.) *Recalcitrant Crop Seeds*. Kuala Lumpur, Malaysia, Tropical Press SDN.BDH, pp. 53−89.

Madhusudanan KN, Babu V. 1994. Recalcitrancy, viability and germination of nutmeg seeds. *Journal of Plantation Crops,* **22**: 25−29.

Ng FSP. 1978. Strategies of establishment in Malayan forest trees. In: P.B. Tomlinson & M.H.Zimmermann (Ed.) *Tropical Trees as Living Systems*. Cambridge, UK: Cambridge University Press, pp. 129−162.

Pammenter NW, Berjak P, Farrant JM, Smith MT, Ross G. 1994. Why do stored, hydrated recalcitrant seeds die? *Seed Science Research,* **4**: 187−191.

Rajeswari Dayal B, Kaveriappa KM. 2000. Effect of desiccation and temperature on germination and vigour of the seeds of *Hopea parviflora* Beddome and *H. ponga*(Dennst.) *Mabb. Seed Sci & Technol,* **28**: 497−506.

Ravi Kumar K, Ved DK. 2000. *One hundred red listed medicinal plants of conservation concern in Southern India.* Bangalore, India: FRLHT.

Roberts EH. 1973. Predicting the storage life of seeds. *Seed Sci Technol*, **1**: 499−514.

Sangakkara UR. 1993. Effect of Time of Harvest and Storage Conditions on germination of Nutmeg (*Myristica fragrans* Houtt), *Journal of Agronomy and Crop Science,* 170: 97−102.

Schmidt L. 2000. *Guide to Handling of Tropical and Subtropical Forest Seed*. Humlebaek, Denmark: Danida Forest Seed Centre, p.511.

Tompsett PB. 1992. A review of the literature on storage of dipterocarp seeds. *Seed Sci & Technol,* **20**: 251−267.

Wesley-Smith J, Vertucci CW, Berjak P, Pammenter NW, Crane J. 1992. Cryopreservation of desiccation-sensitive axes of *Camellia sinensis* in relation to dehydration, freezing rate and the thermal properties of tissue water. *J Plant Physiol*, **140**: 596–604.